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A PRIMER FOR THE APHID HUNTER.

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The objectives of the aphid hunter may be said to be only partially achieved when he returns to the laboratory with the trophies of his hunt, for there still remains the task of mounting the specimens if they are to be permanently preserved.

The collection of material calls for some knowledge of how and where to hunt if the collecting is to be done efficiently. If the minimum of labor is to be expended certain equipment is necessary, or at least desirable; and if ideal slides are desired, (and who wishes inferior ones), the aphids must be correctly mounted.

For convenience, I have divided the Aphid Hunter's Primer into several sections—the first of which will be devoted to equipment.

The collecting equipment need not be extensive. It is assumed that the collector will usually use a car, not only as a means of locomotion but as a center from which to make numerous short side-trips into desirable collecting territory while on a more extensive trip. By using a car as a base it will not be necessary for the collector to burden himself down with containers and collecting equipment necessary to last all day unless definite provisions for such extended periods away from the car are made.

We may list essential equipment as follows: A knapsack or other suitable container for the remaining equipment. For aphid containers the writer prefers large glass vials with spring metal caps. Glass containers permit frequent observation without endangering loss of the contents. They do not rust and are easily kept clean. Danger of loss by breakage is objectionable but occurs infrequently. Having all of one's containers of uniform size has obvious advantages. Cigarette tins, tobacco cans, salve boxes and seal tight containers of various sizes have ways of finding themselves in the aphid collector's kit, and serve excellently if one does not object to miscellaneous containers. At first glance containers of various sizes appear to have their points of superiority, for a container may be selected to

fit the desired amount of material of a given species. The writer has found it better to use as many small containers as necessary for a given species rather than to risk having something happen to the aphids in a single large container. Uniform containers are also much easier to pack. A good stout knife is essential for cutting twigs, trimming leaves, or removing sections of bark upon which aphids may be feeding. Some collectors will prefer not to bother with live material and such will place their material in vials containing seventy per cent alcohol. This method is perhaps best when one is limited for time, or when there is likelihood of the aphids not surviving the return trip to the laboratory; for once in alcohol the aphids need no further immediate attention. Such collectors will find it advantageous to partially fill the vials with alcohol before leaving the laboratory. Slides made from material preserved in alcohol are satisfactory when properly mounted but less attractive than slides mounted from freshly processed material. Hence the collector desiring the best slides possible will endeavor to keep his material alive until mounted.

Inasmuch as aphids occasionally occur on the roots of plants and probably occur more frequently than realized, a small trowel or chisel will be found most helpful in the collector's kit if subterranean forms are to be collected.

Aphids in containers appear to be quite easily affected by heat, especially when the car has to stand in the sun for various periods of time. The effect by heat may be somewhat delayed by packing the filled containers in grass in a large container, such as a bushel basket, and then covering it well.

Because aphids are so intimately associated with their hosts and because the host's name is highly desirable on a correctly labeled slide, the host will either have to be recorded on a slip of paper placed in the vial or the vial given a number corresponding to a number given the unknown host, which must then be collected and pressed so that it may be determined at some future time. Plants dry rapidly in arid regions so should be pressed as soon as possible. A Saturday Evening Post makes an ideal temporary plant press, being approximately the size of regulation drying blotters.

Some aphid hunters will prefer to carry and use a large sweep net. From time to time the collector may desire to beat certain species of plants in order to quickly determine the presence or absence of aphids on them. A net is especially useful when collecting aphids which occur singly or when collecting on conifers. It is the only efficient manner in which to collect Essigella. A small notebook in which to record such data and observations as are desired, a hand lens, a pair of forceps and a camel's hair brush complete the aphid hunter's essential equipment.

The second section of the primer will be devoted to notes regarding where to collect.

Where and how the aphid hunter collects will depend upon his objectives. If his object is merely to make an aphid collection or to add to a list, he may follow some such procedure as here suggested.

Assuming that he is familiar with the flora in the region in which he is

to hunt, let him make a list of the aphids known to frequent hosts found in that region by consulting such host plant indices as Wilson's or Davidson's or, lacking access to these, let him consult regional indices, such as those found in the aphid works of Oestlund, Williams, Sanborn, Patch, Swain, Essig, Gillette and Palmer, Knowlton or the Aphiidae of Illinois. Then if he has access to aphid literature, let him acquaint himself with the habits of the various species likely to occur in his collecting territory so that he will not look on leaves or branches for species which occur on roots. Thus one might say that the collection of the aphid slide begins in the library. Certainly by following such a procedure the inexperienced collector may save himself hours of time in the field and almost assure himself satisfactory results. The collector desiring to merely add known species to a given list need only confine his searching to those hosts whose representative species are absent from his list or collection.

The collector desiring to discover new species must modify the procedure just given. For example, he may make a list of the plant species growing in his territory from which few or no aphids have been described and confine his searching for a time to these. This procedure produces results, but is often discouraging if continued for long. One who collects for new species must be content to take or at least observe many known forms in proportion to the species which he takes for new. He should, first of all, free himself from professional prejudices. If he would find forms overlooked by others, he must use unconventional methods; he must look on and where others before him have not first looked. Secondly, he must examine all plants for aphids even those which he considers too insignificant to harbor them. He must examine regions of plants from which aphids have not been described. Nor can the new species hunter confine his searching to a given host species growing in a given ecological habitat, for certain aphids show a preference to hosts growing in shaded areas, in open spaces, or in moist situations. Thus by confining his collecting to plants growing in a given restricted area, the aphid hunter is almost sure to reduce the number of his findings. By using these tactics the one whose objective is finding new species may even invade a territory seemingly posted not to have new species and achieve success.

The aphid collector will find it well to have a working understanding of the different ecological and geological regions of his state or of the areas within which he wishes to collect. The advantage of such information is most obvious to any insect collector but is especially important to the collector of aphids, for different ecological and geological areas are sure to mean different types of vegetation which in turn is indicative of possible aphid hosts. Thus we see that by carefully planning one's itinerary and objective before starting to collect that much time can be saved in the field and the success of the expedition almost assured.

Neither the professional collector nor the amateur hunter can ignore regions near to his home or office. The back yard, the neighbor's weed patch, the campus, an old cemetery, a railroad right-of-way or a nursery may be an excellent site in which to collect whenever time is limited.

The third section will deal with the subject what and how to collect.

It is a decided advantage to have both alate and apterous forms available for study. Lacking both forms, it is best to have alate specimens in most groups; not only because the alate forms possess the best diagnostic characters but because most keys are based on them. The time will no doubt come, if it is not already here, when immature forms will be necessary in the collection of one whose interest lies in a study of the phylogeny of the group. However, alate and apterous forms are not always present at the time the collector makes his visit. If the collector is so situated that he may conveniently return to the place at a future time, he need only take such forms as are available and mark the spot so that it may again be located.

If the material is especially rare or valuable, the collector may care to enclose a twig or branch of the host by means of a celluloid "lamp chimney" closed at both ends by muslin sleeves, which may be tightly tied after the chimney is in position over the desired region. When this method is employed, care must be exercised to see that all insect aphid hunters and their eggs have been removed. Another objection to cages is that they are quite conspicuous and for that reason rather impractical in frequented regions.

If it seems improbable that the collector will return to a particular site again, he may take some of the available forms alive with the hope of establishing them on similar hosts near at home, or he may consider transplanting the hosts to a site in his own garden. This procedure is impractical for most plants but has been tried successfully by Gillette and Palmer with conifers. Twigs of conifers can withstand relatively long periods of time with their proximal ends submerged in water or "planted" in damp sand. By resorting to the above methods most species of the genus Cinara can be persuaded to produce several generations. Such twigs, however, must be kept in a rather cool place, otherwise the spirit of the wanderlust gets the best of the aphids and they start going places.

There is a great paucity of knowledge regarding the sexual forms of the aphids and therefore collecting in the late fall for these forms should greatly increase our knowledge of these insects. Sexual forms are especially desirable in certain groups if the worker is interested in taxonomic divisions larger than genera, or in phylogeny.

Having located aphids which one desires they may be removed from the host directly into vials or other containers by means of a forceps or a small camel's hair brush, or small sections bearing aphids may be cut from the host. Sections of bark removed from conifers are inwardly sticky and care should be used to wedge such sections with the raw side to the edge of container so as to minimize the danger of the aphids mounting themselves in the natural balsam. Ordinarily it will be well to place some of the host into the container, not only to furnish food but a resting surface for the aphids until one is ready to mount them. The host material and aphids will cause the inside of the container to sweat, especially in summer. To prevent this, strips of blotting paper or bits of tissue paper should be placed in the container to absorb the excessive moisture. Care should be taken not to place too much material in a container and also to see that all aphid

feeders have been removed. It is also well to collect a little more material than one thinks is enough so that only the best need be used. Live material under ordinary conditions does not stand up for long. It is useless to spend time collecting more material than can be mounted at the end of a day's collecting unless one can slip his containers into the family refrigerator, or is satisfied with material preserved in alcohol.

The next section will be devoted to the various mounting media and the preparation of the aphids for mounting.

Before describing the preparation of the ideal aphid slide, it may be well to define what we mean by the term. First of all, it is assumed that representative adult alate and apterous forms are available so that both forms may be placed on the same slide. It is further assumed that the specimens are mounted in positions favorable for study and that the characters necessary for classification are clearly visible when observed under the microscope. It is further assumed the ideal slide will be properly labeled as to host, locality, date, and name of collector as well as the name of the person responsible for the determination of the genus and species. It might as well be stated first as last that no one method of treatment and no one mounting medium can be relied upon to give satisfactory results for all forms, and the aphid technician will have to vary his treatment and procedure accordingly. The choice of mounting medium will depend somewhat upon the results desired, the time available at the time the slides are made and the prior treatment of the specimens to be mounted.

Canada Balsam is perhaps the most universal mounting medium used by aphid technicians. There is no question of its permanency. Live aphids mounted in it clear only after a relatively long time, an objectionable feature if the slides are to be studied soon after being made. Such slides, even though sometimes badly stained, are serviceable, if not attractive. Canada Balsam is sometimes rather darkly tinted—a factor objectionable to some. It sets or hardens slowly—a rather disagreeable characteristic if slides are to be studied or transported soon after being made. Slides made from processed material or material which has been preserved in strong alcohol are immediately ready for study but the slides are soft and must be handled with care.

The writer prefers to use either Gum Damar or Euparal as a mounting medium and the choice between these depends entirely upon the prior treatment accorded the aphids and the time available when the slides are made.

Lee in his Vade Mecum speaks rather slurringly about the permanency of slides mounted in Gum Damar. This, I think, is rather unfounded. I have seen slides of aphids mounted in Gum Damar by Oestlund many years ago, which are as fresh looking as the day they were made. Gum Damar is absolutely worthless as a mounting medium for unprocessed aphids, for such material will not clear regardless of the amount of time allowed. Alcoholic material must be thoroughly dehydrated before mounting; otherwise it clears very slowly. It is the writer's first choice for processed material. The fact that it sets and hardens quickly makes it an

especially desirable mounting medium when the slides are to be used or transported soon after they have been made. It has little or no color.

Euparal is perhaps the best all around mounting medium. Like Gum Damar, it sets and hardens quickly, and is comparatively free from color. Live aphids may be mounted in it and clear in a shorter time than if mounted in Canada Balsam. Such slides are, however, open to the same objection as those mounted alive in Canada Balsam. Processed aphids need not be so thoroughly dehydrated as those intended for mounting in Gum Damar. Specimens in alcohol may be mounted directly from seventy per cent alcohol and the slides be cleared and ready for study within a week. Many of the early aphid workers used glycerine as a mounting medium. Glycerine makes a satisfactory temporary mounting medium, but should not be employed for permanent mounts even though these are ringed. Buxton's mounting medium, a mixture of water, glycerine, gum arabic and chloral hydrate in definite proportions, appears to be undesirable for mounting aphids. Slides made with it clear poorly, often crystallize and sometimes lose their cover slips and have to be remounted within a few years.

Now let us consider the preparation of an aphid slide. Most species of aphids will respond favorably to the following treatment: Kill aphids by pouring boiling hot 95% alcohol over them, perhaps allowing the boiling to continue for a second or two. Large, thick skinned aphids are best killed in boiling hot water and then transferred to hot 95% alcohol. After they have been killed they are allowed to stand in 95% alcohol for a short time, after which they are transferred to absolute alcohol. If large, they are punctured with a pin. From the absolute alcohol they are transferred to xylol from which they are mounted. No exact time can be given for each of the above stages in the process because the time will largely be governed by the peculiarities of the individual species. In general, it will be safe to allow as much time to clear and dehydrate as necessary without rendering the aphids and their appendages brittle. The entire process need not require more than fifteen to twenty minutes. After the aphids have been in xylol for a very brief period, the clearing process may be speeded up by the use of oil of wintergreen as a clearing agent. When using oil of wintergreen, however, care must be taken not to allow it to act too long as it quickly renders aphids too brittle. Some aphids after reaching the xylol will cause it to turn "milky." When this occurs the aphids should be transferred back to alcohol for further dehydration. Boiling for a second or two in 95% alcohol is usually sufficient to bring about the desired result. Color is more or less perfectly preserved by this procedure.

Small 50 cc. beakers are used for heating the alcohol with which to kill the aphids and only a limited amount (25–30 cc.) is heated at a time. The aphids are afterwards transferred to Syracuse watch glasses. Several species may be treated at the same time so that once the process is started work may continue without interruption. When many species are treated at the same time it will be necessary to carry the data slip through each stage along with the aphids so as to avoid the possibility of a mixup.

Some workers may object to the use of heat for killing because it compli-

cates the process of mounting, if this is to be done away from the home base. However, canned heat may be employed when away from a source of electricity and an electric grill or toaster when electricity is available. Thus aphids may be mounted in a hotel room or mountain cabin. Processed aphids retain original color more or less perfectly.

Aphid technicians have thought it necessary to boil or at least soak certain species in potassium hydroxide solution in order to bring out waxpore plates, or hair distribution on the abdomen. This is at best a drastic procedure. The integument of the forms needing this process most is often very thin and there is danger of the potassium hydroxide being allowed to act too long. Aphids treated with potassium hydroxide must be run carefully through a graded series of alcohols before mounting, in order to harden the chitin and prevent the collapsing of small parts. Inasmuch as the potassium hydroxide has for its object chiefly the destroying of pigment, the writer has found the mouth wash Zonite satisfactory for this purpose, after which the specimens may be dehydrated. This method has little or no effect on tissue and bleaches well.

W. Roepke's directions for treating aphids for microscopic study takes them through 70% alcohol, then 75% lactic acid heated in a hot water bath for twenty minutes. From this they are placed in a mixture of equal parts of chloral hydrate and carbolic acid crystals and kept hot in a water bath for twenty minutes or longer if necessary. I have not tried his methods but have seen beautiful slides prepared by Professor Palmer, in which his method was employed. Miss Palmer's criticism is that the method requires too much time. Roepke recommends Berlese fluid as a mounting medium, but as this is quite similar to Buxton's mounting medium which has proved unsatisfactory for aphids, Miss Palmer mounted her specimens in Canada Balsam with satisfactory results after, however, washing the specimens in xylol. Professor Palmer objects to this method because there is some danger of the doubling up and distorting the bodies of the aphids. In this respect it is quite similar to potassium hydroxide solution.

Professor Palmer has found the following procedure successful: Kill in boiling alcohol, dehydrate with high per cent alcohol, then put them on a cover glass and cover with a drop of clearing mixture (made of equal parts of carbolic acid and turpentine). Allow the clearing mixture to act for about fifteen minutes, then mount in Balsam. Color is more or less perfectly preserved by this method.

Recently Johannsen has recommended 2–4 Dioxane as a clearing agent for histological preparations. This material was tried out on a limited number of aphid species with highly satisfactory results. Small amounts of 2–4 Dioxane were placed in glass covered containers. The odor of 2–4 Dioxane is somewhat objectionable and covered containers were used to lessen the amount freed in the room. The aphids were killed by being placed in unheated, undiluted 2–4 Dioxane and allowed to remain in it for various periods of time. The material did not collapse the appendages, nor shrink the specimens in the least, nor did it affect the color.

Tests showed that specimens could be mounted directly from the 2–4 Dioxane after five minutes' treatment and the slides remain clear. However,

it should be pointed out that these were fall aphids whose bodies likely contained less water than aphids taken at other seasons of the year. The appendages could be manipulated after the specimens had been in the solution for twenty minutes but became quite stiff and brittle if allowed to remain for a longer period of time. The limited number of species on which 2–4 Dioxane has been used and the fact that the specimens were hardly typical does not permit an unqualified endorsement. However, it would appear that if present results can be duplicated that all other dehydrating media and processing methods would be rendered obsolete. Hence, much of the material treated in this section may be regarded as a sort of premature obituary.

The inevitable result of preserving aphids in alcohol is the drying up of the vials and the loss of the material thus preserved. Because such material may often be valuable, it may be worth while to give two procedures by which it may be salvaged.

Almost all of the Thomas collection of Illinois aphids was preserved in alcohol. The time between 1878 and 1928 was more than ample for the contents of the vials to dry up. Inasmuch as most of this was typic material a method by which it could be made available for study was much to be desired. After some consideration the following plan was put in operation: The vials were filled with a weak solution of potassium hydroxide. This softened the chitin and after a short time the aphids had assumed their natural form and except for loss of many appendages were none the worse for having been dried. All that remained to be done was to run the aphids through successive stages of alcohol and mount. The slides were usable and satisfactory. I mentioned the above procedure to my uncle, Professor Chas. F. Hottes, and he suggested that I try a mixture used by botanists to soften and prepare certain kinds of dried botanical material for sectioning. This material called lacto phenol is made as follows: 20 grams melted crystals of carbolic acid c.p., 40 grams lactic acid spec. gravity 1.25, 20 cc. distilled water. This mixture constitutes the concentrated stock solution.

Fortunately I had abundant dried material available for experimental purposes. One part stock solution to two parts of distilled water was tried as a softening agent. This strength worked well on small and medium sized aphids, completely plumping and relaxing them in a remarkably short time. Larger forms did not respond well unless placed in a solution made of 50% stock solution and 50% distilled water.

Botanists use this material cold. I tried keeping the material warm for some specimens and cold for others and achieved equally satisfactory results with both methods. Specimens kept in the stronger mixture clear more completely than those kept in the weaker solution. Specimens, especially small forms and those whose chitin is relatively thin, plump within twenty-four hours, some even plumping within twenty minutes. Air bubbles are objectionable and I found it best to treat aphids with the mixture till all of these had been destroyed. The time required for this was from three hours to four days. The material was then taken from the lacto phenol mixture, washed in absolute alcohol (I question the necessity

of this) and mounted. Slides made by this procedure are uniformly good. I next tried dehydrating live aphids and then treating them with lacto phenol. Yellow aphids treated thus when mounted retained their yellow color; red aphids did not. Nevertheless, such slides were about as good as any slides in my collection made from unprocessed aphids. Lacto Phenol treated aphids can not be sectioned to show internal structures but from the standpoint of whole mounts it can be recommended without qualification. In fact, I am seriously considering dehydrating some of my next season's catch and working it up at my leisure next winter without the fuss and bother of using heat, alcohol and xylol during the collecting season if 2–4 Dioxane upon further experimentation proves unsatisfactory.

The method of mounting aphids on a slide is always the same regardless of the prior treatment accorded them or of the mounting medium used. Only standard sized slides should be used for mounting aphids. The cover slips should be of noncorrosive glass of No. II thickness. Whether round or square cover slips are used is a personal matter. The size of cover slips is optional but it is well to have two sizes, one 12 mm. for single specimens and 22 mm. for use when several specimens are to be mounted under the same cover.

The process of making a slide is at best a sticky one. Therefore, I prefer to have several sheets of white paper on my table top. These may be conveniently held in place by means of a thumb tack. It is then a simple matter to pull off the top sheet when it becomes soiled or sticky. A slide is roughly outlined in a convenient place on the paper and the same procedure is followed with a cover slip somewhere near the center of the outline of the slide. This easily made diagram insures the proper orientation of the cover slip on the finished slide. Slides are placed in the outlined area and the aphid to be mounted on the slide selected and placed within the area representing the cover slip.

Excess fluid may now be removed by means of a strip of blotting paper if the aphids have been processed. The proper amount of mounting medium is now placed on the aphids and the process of orientation of specimens begun with dissecting needles. If several aphids are to be mounted on the same slide it will be well to mount some specimens with the ventral surface up so that the beak may be observed without turning the slide over. Single specimens should always be mounted with the dorsal surface uppermost. Care should be taken to spread the wings if the specimens are alate and not to break off the legs and antennae. Attempts should be made to pull the antennae and legs away from the body so that they may be observed and measured and not obscured by it. If bubbles form during the orientation process they may be easily punctured by placing the needles into the solvent of the mounting medium and then placing them while still wet into the bubbles. After the aphids are in position a cover slip is placed so that one side of it is in contact with the mounting medium and gently allowed to fall. If bubbles form they will be carried to the opposite side of the cover slip where they may be easily punctured without again lifting the glass. If the cover slips are placed directly from the top bubbles are sure to form and being near the center will require that

the glass be lifted before they may be destroyed. If not enough mounting medium was used in the first place more may be added from the side after the cover slip is in position. The cover slip should be parallel with the surface of the slide. Aphids may be placed in strategic points to hold the cover slip level or small bits of broken slide may be used as props to hold the cover slip up. If one wishes especially nice slides he may mount his aphids within horn rings such as used by bacteriologists in hanging drop studies. If rings are used, however, care must be used to fill the ring to overflowing before placing the cover slip so as to avoid bubbles.

Ordinarily one does not take time to completely label slides at the time they are made, hence the slides are usually given a number with a grease pencil, this number corresponding to data recorded in an accession book.

The freshly made slides are then placed in a slide box which is kept in a vertical position until the mounting medium has had time to set.